Associated Content

Supplementary Table 1. UPLC/MS analysis methods

Supplementary Table 2. "ST2A" page containing descriptive, analytical, and statistical data obtained for metabolite variables included in the analysis. (Columns from left to right) Variable identifier; Analytical platform; Metabolite Class; Retention time; Ion; *m/z*; Metabolite identification method; Metabolite identification detail 1; Metabolite identification detail 2; Optimal internal standard; % of study data points outside linear detection range; QC Validation %CV batch 1; QC Validation %CV batch 2; QC Validation %CV batch 3; QC Validation %CV batch 4; QC Validation %CV batch 5; QC Validation %CV batch 6; QC Validation %CV inter-batch; ion abundance ratio steatosis/normal liver – lean/pre-obese cohort; ion abundance ratio steatosis/normal liver – lean/pre-obese cohort (p-value); ion abundance ratio NASH/normal liver – lean/pre-obese cohort; ion abundance ratio NASH/normal liver – lean/pre-obese cohort (p-value); ion abundance ratio NASH/steatosis – lean/pre-obese cohort; ion abundance ratio NASH/steatosis – lean/pre-obese cohort (p-value); random forest variable importance score - lean/pre-obese cohort; ion abundance ratio steatosis/normal liver – obese class I-II cohort; ion abundance ratio steatosis/normal liver – obese class I-II cohort (p-value); ion abundance ratio NASH/normal liver – obese class I-II cohort; ion abundance ratio NASH/normal liver – obese class I-II cohort (p-value); ion abundance ratio NASH/steatosis – obese class I-II cohort; ion abundance ratio NASH/steatosis – obese class I-II cohort (p-value); random forest variable importance score - obese class I-II cohort; ion abundance ratio steatosis/normal liver – obese class III cohort; ion abundance ratio steatosis/normal liver – obese class III cohort (p-value); ion abundance ratio NASH/normal liver – obese class III cohort; ion abundance ratio NASH/normal liver – obese class III cohort (p-value); ion abundance ratio NASH/steatosis – obese class III cohort; ion abundance ratio NASH/steatosis – obese class III cohort (p-value); random forest variable importance score - obese class III cohort. "ST2B" page containing significant variables differentiating between steatosis and NASH patients. (Columns from left to right) Variable identifier; Metabolite Class; Metabolite identification detail 1; Metabolite identification detail 2; Observations: Patient cohort in which the variable was significant.

Supplementary Table 3. Cumulative distribution function of the Inter-batch %CV for each platform.

Supplementary Figure 1. (left) Raw response curves for standard compounds representing metabolite classes included in the analysis **(a-r)** Platform 1 – free sphingoid base, sphingosine-1-phosphate **(a)**; Platform 1 – oxidized fatty acids, 15-HETE **(b)**; Platform 1 – acyl carnitines, AC(3:0) **(c)**; Platform 1 – monoacyl-, monoetherglycerophosphoethanolamine, PE(14:0/0:0) **(d)**; Platform 1 – nonesterified fatty acids, FFA(19:0) **(e)**; Platform 1 – Bile acids, dehydrocholic acid [M-H] **(f)**; Platform 1 – Bile acids, dehydrocholic acid [M+CO_2H] **(g)** Platform 1 - monoacyl-, monoetherglycerophosphocholine, PC(13:0/0:0) **(h)**; Platform 2 – amino acids, tryptophan-d_5(indole-d_5) **(i)**; Platform 3 – ether-acyl-, diacylglycerophosphocholine, PC(19:0/19:0) **(j)**; Platform 3 – triacylglycerides, TG(13:0/13:0/13:0) **(k)**; Platform 3 – sphingomyelin, SM(d18:1/6:0) **(l)**; Platform 3 – ceramides, Cer(d18:1/17:0) **(m)**; Platform 3 – cholesterol esters, ChoE(12:0) **(n)**; Platform 3 – ether-acyl-, diacylglycerophosphoethanolamine, PE(17:0/17:0) **(o)**; Platform 3 – diacylglycerophosphoinositol, PI(18:0/20:4) **(p)**; Platform 3 – diacylglycerol, DG(18:1/20:0) **(q)**; Platform 3 – monohexosylceramides, CMH(d18:1/16:0) **(r)**. (right) Box plots showing the response distribution of included study data points for all detected variables belonging to a given metabolite class **(a-r)**.

Supplementary Figure 2. Heat map representation of the serum metabolic profile obtained from patients included in the study estimation group: normal liver subjects (left), steatosis patients (middle), and NASH patients (right). Each data point

corresponds to the relative ion abundance of a given metabolite (vertical axis) in an individual patient's [horizontal axis, ordered by BMI as depicted by **(a)**: green, lean/pre-obese cohort (BMI < 30 kg/m^2); orange, obese class I-II cohort (BMI 30-40 kg/m^2); red, obese class III cohort (BMI > 40 kg/m^2)] serum extract. The scale is defined by panel **(b)**, where colors from green to red show, for each metabolite, its relative ion abundance in the serum extract of a given subject with respect to that found in the rest of the study population, as represented by the 10th-100th percentiles.

Supplementary Figure 3A-3K. Heat map representations of serum metabolic profile obtained from patients included in the study estimation group. **(a)**, **(b)**, and **(c)** metabolite ion abundance ratios in BMI cohorts: lean/pre-obese (bottom), obese class I-II (middle), and obese class III (top), comparing histological groups: steatosis/normal liver, NASH/normal liver, and NASH/steatosis respectively. For each comparison, log transformed ion abundance ratios are depicted, as represented by the scales (d), where pronounced colors correspond to significant ($p<0.05$ – two-tailed Wilcoxon Rank Sum Test) changes, and (e) where light colors correspond to non-significant ($p>0.05$ – two-tailed Wilcoxon Rank Sum Test) changes. **A:** Glycerolipids: diacylglycerides (DG - left) and triacylglycerides (TG - right). The nomenclature *C:D* is used, where *C* is the total number of carbon atoms and *D* is the total number of double bonds in the acyl chains esterified to glycerol. Supplementary Table 2 contains details on the constituent acyl chains, as determined from the MS/MS spectra of each lipid. **B:** Cholesterol esters (ChoE - left) and bile acids (right). For the ChoE species the nomenclature *C:D* is used, where *C* is the total number of carbon atoms and *D* the total number of double bonds in the acyl chain esterified to cholesterol. **C:** Sphingolipids, left to right: sphingomyelin (SM), ceramides (Cer), free sphingoid bases, monohexosylceramides (CMH). For SM,

Cer, and CMH species the nomenclature *dA:B/C:D* is used, where *dA:B* represents the sphingoid base: d18:1, sphingosine; d18:2, sphingadiene; d18:0, sphinganine, and *C:D* the number of carbon atoms *C*, and double bonds *D*, contained in the *N*-linked fatty acid. **D:** Diacylglycerophosphocholine (left) and ether-acyl-glycerophosphocholine (right). The nomenclature PC(*A:B*/*C:D*) is used, where *A:B* and *C:D* refer to the number of carbon atoms:number of double bonds contained in the *sn*-1 and *sn*-2 side chains respectively. For ether-acyl-glycerophosphocholine species the prefix, O-, denotes the presence of an alkyl ether substituent. The suffix, e, indicates the presence of an ether linked substituent with one or more double bonds. For both diacylglycerophosphocholine and ether-acyl-glycerophosphocholine species composite nomenclature, referred to as the sum of fatty acid pairs (i.e. PC(*X:Y*), where $X = A+C$ and $Y = B+D$), was used where evidence was found for the contribution of multiple species to a single chromatographic peak [e.g. PC(32:1) = PC(16:0/16:1) + PC(14:1/18:0) + PC(14:0/18:1)]; Supplementary Table 2 contains details on the constituent fatty acid pairs, as determined from the MS/MS spectra of each lipid. **E:** Monoacylglycerophosphocholine (left) and monoetherglycerophosphocholine (right). Nomenclature follows that used in panel 3D. **F:** Diacylglycerophosphoethanolamine (left) and ether-acyl-glycerophosphoethanolamine (right). Fatty acid constituent nomenclature uses the same notation as panel 3D. **G:** Monoacylglycerophosphoethanolamine (left) and monoetherglycerophosphoethanolamine (right). Fatty acid constituent nomenclature follows that used in panel 3D. **H:** Diacylglycerophosphoinositol. Fatty acid constituent nomenclature uses the same notation as panel 3D. **I:** Nonesterified fatty acids (NEFA-left) and oxidized fatty acids (right). For NEFA species the nomenclature *C:Dn-x* is used, where *C* is the number of carbon atoms, and *D* is the number of double bonds in

the fatty acid chains. A double bond is located on the *x*th carbon-carbon bond, counting from the terminal methyl group towards the carbonyl carbon. For the oxidized fatty acids, HETE denotes hydroxy-eicosatetraenoic acids, HODE denotes hydroxyl-octadecadenoic acids, and OxoODE denotes oxo-octadecadenoic acids. **J:** Amino acids (left) and Acyl carnitines (right). **K:** Unidentified variables with tentative structural assignments obtained from accurate mass (tolerance = 5ppm) online database (http://www.hmdb.ca/) searching, left to right: nonesterified fatty acids (NEFA), oxidized fatty acids [HETE denotes hydroxy-eicosatetraenoic acids, EET denotes epoxy-eicosatetraenoic acids, DiHETrE denotes dihydroxyeicosatrienoic acids, HODE denotes hydroxyl-octadecadenoic acids, and OxoODE denotes oxo-octadecadenoic acids], miscellaneous database hits – platform 1 (see Supplementary Table 2 for database hits), miscellaneous database hits – platform 3 (see Supplementary Table 2 for database hits).